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# Effect of induction of subacute ruminal acidosis on milk fat profile and rumen parameters

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## ABSTRACT

High-concentrate diets can lead to subacute ruminal acidosis and are known to result in changes of the ruminal fermentation pattern and mammary secretion of fatty acids. The objective of this paper is to describe modifications in milk fatty acid proportions, particularly odd- and branched-chain fatty acids and rumen biohydrogenation intermediates, associated with rumen parameters during a 6-wk subacute ruminal acidosis induction protocol with 12 ruminally fistulated multiparous cows. The protocol involved a weekly gradual replacement of a standard dairy concentrate with a wheat-based concentrate (610 g of wheat/kg of concentrate) during the first 5 wk and an increase in the total amount of concentrate in wk 6. Before the end of induction wk 6, cows were switched to a control diet because 7 cows showed signs of sickness. The pH was measured continuously by an indwelling pH probe. Milk and rumen samples were taken on d 2 and 7 of each week. Data were analyzed using a linear mixed model and by principal component analysis. A pH decrease occurred after the first concentrate switch but rumen parameters returned to the original values and remained stable until wk 5. In wk 5 and 6, rumen pH values were indicative of increasing acidotic conditions. After switching to the control diet in wk 6, rumen pH values rapidly achieved normal values. Odd- and branched-chain fatty acids and C18:1 *trans*-10 increased with increasing amount of concentrate in the diet, whereas C18:1 *trans*-11 decreased. Four fatty acids [C18:1 *trans*-10, C15:0 and C17:0+C17:1 *cis*-9 (negative loadings), and *iso* C14:0 (positive loading)] largely correlated with the first principal component (PC1), with cows spread along the PC1 axis. The first 4 wk of the induction experiment showed variation across the second principal component (PC2) only, with high loadings of *anteiso* C13:0 (negative loading) and C18:2 *cis*-9,*trans*-11 and C18:1 *trans*-11 (positive loadings). Weeks 5 and 6 deviated from PC2 and tended toward the negative PC1 axis. A discriminant analysis using a stepwise approach indicated the main fatty acids discriminating between the control and acidotic samples as *iso* C13:0, *iso* C16:0, and C18:2 *cis*-9,*trans*-11 rather than milk fat content or C18:1 *trans*-10, which have been used before as indicators of acidosis. This shows that specific milk fatty acids have potential in discriminating acidotic cases.
**Key words:** subacute ruminal acidosis, milk fat profile, rumen parameter

## INTRODUCTION

Subacute ruminal acidosis is a digestive disorder indicated by symptoms that are subtle, nonexclusive, and often delayed from the time of incidence (Enemark, 2008). Rumen pH parameters are the only reliable tools to diagnose SARA (Keunen et al., 2002), although ruminal pH varies considerably at different locations in the rumen and during the day. As a result, rumen pH conditions that define SARA are still an issue and various parameters and associated threshold values have been proposed. Time or area (time × pH) below a certain pH as calculated from continuous pH monitoring seems to be a potential indicator (Keunen et al., 2002; Dragomir et al., 2008), with thresholds of 283 and 475 min of pH below 5.6 or 5.8, respectively (AlZahal et al., 2007). Further, Dragomir et al. (2008) concluded that the rate of pH decline or the time within which pH reaches its minimum are useful alternative indicators of the ruminal status. Currently, only 2 techniques are available to measure rumen pH under field conditions: rumenocentesis and oral stomach tube insertion (Duffield et al., 2004). Continuous pH registration recorded by a swallowed probe is under investigation, but economic feasibility and practical issues, such as calibration of the pH equipment, impair its use as a diagnostic tool in dairy herd management.

Accordingly, there is increasing interest in milk compounds (e.g., milk fatty acids; **FA**) as potential diagnostic tools of rumen function (Vlaeminck et al., 2006a). Milk odd- and branched-chain FA (**OBCFA**)

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are of particular interest in this respect because they have been linked to the rumen fermentation pattern (Vlaeminck et al., 2006b; Cabrita et al., 2007; Craninx et al., 2008). Moreover, accumulation of FA during rumen biohydrogenation depends on the rumen environment, with predominant switches toward the C18:1 *trans*-10 pathway at low ruminal pH (e.g., Enjalbert et al., 2008). Because of these relationships and the ease of obtaining milk samples, monitoring the FA profile of milk has potential value in the diagnosis of SARA and could facilitate its prevention. However, relationships between milk FA profile (including OBCFA) and rumen fermentation (rumen pH and VFA proportions) have not yet been determined in experiments designed to induce SARA. The objective of this study was to establish relationships between classical SARA, indicators in the rumen, and milk FA during a 6-wk experiment in which rumen function was challenged weekly by increasing amounts of rapidly fermentable carbohydrates. To our knowledge such a detailed combined report has not previously been published.

## MATERIALS AND METHODS

### Experimental Design and Animals

Twelve multiparous ruminally fistulated Holstein-Friesian dairy cows were used in a complete randomized block design. Cows were paired according to milk yield and stage of lactation and allocated at random to treatment A or B. Cows in treatment A received a control diet, whereas cows in treatment B received a supplementary mixture of feed additives (yeast, vitamin E, and buffer) aimed at preventing SARA. Average (mean ± SD) DIM, milk yield (kg/d), and BW (kg) at the beginning of the experiment were 236 ± 45, 40 ± 4, and 701 ± 41, respectively, for treatment A and 227 ± 47, 38 ± 7, and 714 ± 33, respectively, for treatment B.

The experiment consisted of preinduction and induction periods. A standard dairy diet was administered during the 3-wk preinduction period. The same diet was given during the first induction week and contained grass and maize silage and the original standard pelleted concentrate. During the following 4 induction weeks, the standard pelleted concentrate was gradually replaced by a pelleted concentrate containing ground wheat to induce mild SARA, but the forage:concentrate ratio remained constant. Finally, the total amount of wheat-based concentrate was increased in wk 6 by 2 to 4 kg/d depending on the cow's milk yield. In this last week cows were switched to a control diet between d 2 and 7 because of signs of clinical acidosis in 7 cows.

Cows were housed in a tie-stall barn (Velddriel, The Netherlands) and the experiment took place from January to March 2007. The barn was illuminated by daylight and by fluorescent lamps, which were turned on during the experimental procedures. This experiment was approved by The Institutional Animal Care and Use Committee of the University of Groningen (Groningen, the Netherlands).

### Experimental Treatments and Diets

The offered diets met the cows' requirements for energy, protein, vitamins, and minerals. A mixture of corn silage and grass silage (67:33 wt/wt; DM basis) was offered at 110% of the preexperimental ad libitum intake. Dairy concentrate was fed at a fixed rate per cow pair. Concentrate level was based on the milk production in the preexperimental period. The amount of concentrate was divided into 2 (at 0700 and 1700 h) or 3 (at 0700, 1700, and 1200 h) meals. Concentrate supply was maximized at morning and evening meals, offered in equal proportions with a limit of 5 kg/meal. Amounts of concentrate in excess of 10 kg were supplied at 1200 h. In wk 6, concentrate supply was limited to the morning and evening distribution and meals of up to 8 kg of concentrate were offered. Any refused concentrate was removed, weighed, and inserted directly into the rumen 1 h after offering. This happened 2 times, on d 7 of wk 3 (13 kg of concentrate put in the rumen of one cow) and d 2 of wk 6 (1.98 kg of concentrate put in the rumen of one cow). These data points showed similar milk FA profile as the rest of the data and were used in further analysis. The standard pelleted concentrate:wheat-based concentrate proportions were 4:0 in the preinduction period and 4:0, 3:1, 2:2, 1:3, 0:4, and 0:4 in wk 1, 2, 3, 4, 5, and 6, respectively. The composition of the wheat-based concentrate and standard pelleted concentrate is given in Table 1. The chemical composition of the grass and corn silage is given in Table 2 and the exact proportions given to 2 extreme pairs (pairs 1 and 6) during the whole induction experiment can be found in Table 3. Rations of the other pairs varied between those extremes. Feed additives (0.33 kg/d) were mixed with ground corn (0.17 kg/d). This mixture was then fed to group B cows together with the roughage. Ground corn (0.17 kg/d) was supplied to the roughage of group A cows. Water was available ad libitum via individual water bowls. However, in wk 4 on d 2 there was an interruption to the water supply of one cow (cow 2) for several hours, which may have influenced results in that week.

### Sample Collection and Measurements

***Feed Analysis and Cow Performance.*** Both corn silage and grass silage were sampled separately

**Table 1.** Ingredients, chemical composition, and nutritive values of the 2 concentrates

| Item | Wheat-based concentrate | Standard dairy concentrate |
|---|---|---|
| Composition (%) | | |
| Corn gluten feed | 7.76 | 18.08 |
| Limestone | 1.37 | 0.37 |
| Molasses | 6.85 | 3.52 |
| Monocalcium phosphate | 0 | 0.15 |
| Rumen-inert fat | 0 | 2.97 |
| Salt | 0.33 | 0.19 |
| Soybean hulls | 0 | 12.59 |
| Soybean meal | 16.15 | 19.23 |
| Protected soybean meal | 5.82 | 3.42 |
| Sugar beet pulp | 0 | 38.38 |
| Vitamin-mineral supplement | 1.08 | 1.11 |
| Wheat | 60.64 | 0 |
| Analysis (per kg of product) | | |
| DM (g/kg of fresh material) | 869 | 896 |
| Ash (g) | 56 | 72 |
| VEM[1] | 961.3 | 999.2 |
| CP (g) | 200 | 202 |
| Crude fat (g) | 16 | 48 |
| Crude fiber (g) | 30.0 | 128.6 |
| Sugar (g) | 67.3 | 68.4 |
| Starch (g) | 368 | 141 |
| NDF (g) | 103 | 313 |
| ADF (g) | 43 | 187 |
| ADL[2] (g) | 8 | 9 |

[1]VEM = feed unit milk. 1,000 VEM = 6.9 MJ of $NE_L$.

[2]ADL = acid detergent lignin.

on the first day of each week, vacuum stored at −20°C, pooled at the end of the experiment, and submitted for analysis by near-infrared reflectance spectroscopy (Blgg Oosterbeek, Oosterbeek, the Netherlands). A single pooled sample of each standard pelleted concentrate and wheat-based concentrate mixture was analyzed by wet chemical analysis (Provimi BV, Rotterdam, the Netherlands). Results included percentages of DM (ISO 6496; ISO, 1999), CP (ISO 16634; ISO, 2008), crude ash (ISO 5984; ISO, 2002), crude fiber, crude fat (Am 5–04; AOCS, 2004), NDF, ADF, and acid detergent lignin; NDF and ADF were corrected for residual ash (Uden et al., 2005). Wheat and sugar beat pulp contained 557 and 5 g/kg of DM starch, respectively, with 52 and 0 g/kg of DM bypass starch, respectively (CVB, 2007). Wheat starch degradation in the rumen was 89.6% in a study by Hindle et al. (2005). Water intake, DMI, and milk yield were recorded daily.

***Rumen Function and Fecal Scoring and Fecal Particle Distribution.*** Rumen pH was measured continuously (5-min intervals) throughout the treatment period using an indwelling pH probe (Sentix 41–3, Boom BV, Meppel, the Netherlands). Probes were calibrated on d 6 of each week and replaced if necessary. Rumen fluid (400 mL) was sampled from the center of the rumen at 1600 h on d 2 and 7 of each week and cooled in an ice-water bath immediately after collection and during processing to stop microbial activity. Samples were mixed thoroughly, strained, and stored in subfractions of 20 mL at −20°C until being analyzed for VFA. Ruminal VFA analysis was performed through separation and quantification by GLC (Shimadzu GC-14A, Shimadzu, Kyoto, Japan) according to Van Ranst et al. (2010). Lactate was measured only on d 2 samples according to the method described by Conway (1962). Fecal consistency was scored visually on the second day of wk 1, 2, 4, 5, and 6 using the 5-point ordinal scale of Zaaijer et al. (2001).

***Milk Analysis.*** Milk samples were collected in the morning and evening of d 2 and 7 of each week. Samples were submitted for fat, protein, urea, lactose, and SCC analyses (MS Nijland, Nijland, the Netherlands) or stored at −20°C until being analyzed for FA composition. Daily average milk proportions of fat, protein, and lactose were calculated from the analytical results, taking into account morning and evening milk production. Morning and evening samples were pooled (50:50 vol/vol) before milk FA analysis by GLC after extraction and methylation as described by Vlaeminck et al. (2005) and were expressed as g/100 g of FA methyl esters (**FAME**). Briefly, milk fat was extracted by the Röse-Gottlieb procedure (ISO 3889; ISO, 2006). After extraction, the solvent was evaporated using a rotary evaporator at room temperature and the extracted lipids were resolved in 20 mL of diethyl ether:petroleum ether (1:1 vol/vol). Tridecanoic acid (Sigma, Bornem, Belgium) was added as the internal standard and FA were methylated with NaOH in methanol (0.5 mol/L) followed by HCl in methanol (1:1 vol/vol; Raes et al., 2001). The FAME were extracted twice with 2 mL of hexane, and pooled extracts were evaporated to dryness under $N_2$. The residue was dissolved in 1 mL of hexane before analysis.

**Table 2.** Chemical composition and nutritive values of corn and grass silage

| Analysis (per kg of DM) | Corn silage | Grass silage |
|---|---|---|
| DM (g/kg of fresh material) | 333 | 688 |
| Ash (g) | 50 | 85 |
| VEM[1] | 934 | 864 |
| CP (g) | 93 | 192 |
| Crude fat (g) | 34 | 35 |
| Crude fiber (g) | 193 | 267 |
| Sugar (g) | <12 | 87 |
| Starch (g) | 310 | — |
| NDF (g) | 403 | 558 |
| ADF (g) | 218 | 308 |
| ADL[2] (g) | 16 | 30 |

[1]VEM = feed unit milk. 1,000 VEM = 6.9 MJ of $NE_L$.

[2]ADL = acid detergent lignin.

**Table 3.** Proportions (pair 1/pair 6; relative to total dietary DM) of corn and grass silage and 2 concentrates for pair 1 and pair 6[1] of a 6-wk acidosis induction experiment

| | Induction week | | | | | |
|---|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | 4 | 5 | 6 |
| Corn | 0.38/0.50 | 0.38/0.50 | 0.37/0.50 | 0.39/0.50 | 0.38/0.49 | 0.38/0.44 |
| Grass silage | 0.19/0.25 | 0.19/0.25 | 0.18/0.25 | 0.20/0.25 | 0.19/0.24 | 0.19/0.22 |
| Standard dairy concentrate | 0.43/0.25 | 0.32/0.19 | 0.22/0.13 | 0.10/0.06 | 0.00/0.00 | 0.00/0.00 |
| Wheat-based concentrate | 0.00/0.00 | 0.11/0.06 | 0.22/0.13 | 0.31/0.19 | 0.43/0.27 | 0.43/0.34 |

[1]Cows were paired according to milk yield and stage of lactation.

***GC Analysis.*** All samples were injected on a GC CP-Sil88 column for FAME (100 m × 0.25 mm × 0.2 μm; Chrompack Inc., Middelburg, the Netherlands) at the following temperature program: start at 70°C for 4 min, then increased at 10°C/min to 150°C, increased at 1°C/min to 165°C, held at 165°C for 20 min, increased at 2°C/min to 170°C, held at 170°C for 10 min, increased at 4°C/min to 215°C, and held at 215°C for 27 min. Two microliters of the sample was injected with a split ratio of 1/50. Because of coelution with C16:1 *cis* and *trans* isomers (Boeckaert et al., 2008; Kramer et al., 2008), *iso* C17:0 and *anteiso* C17:0 were left out of further analyses.

### Statistical Analysis

Cow performance variables, rumen function variables (rumen pH variables, total concentration of VFA, and molar proportions of acetate, propionate, and butyrate) and milk composition variables (protein and fat, g/kg of milk and FA, g/100 g of total FAME) were compared using the linear mixed model for wk 1 to 5 for d 2 (beginning of the week) and d 7 (end of the week) separately: $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term. Week 6 measurements were excluded from the general model as cows were switched to a control diet between d 2 and 7 of wk 6 because of clinical acidotic signs of 7 cows.

Means of d 2 and 7, based on wk 1 to 5, were compared according to the linear mixed model $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term.

Day 2 and d 7 of wk 6 were compared according to the linear mixed model $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

Principal component analysis (**PCA**) was performed based on rumen parameters [pH minimum, pH maximum, pH decrease per hour, area under the curve (**AUC**) pH <5.6 or 6.0, time pH <5.6 or 6.0], milk fat percentage [milk fat, molar acetate, butyrate, and propionate proportions, total VFA (mmol/L)], and specific milk FA (g/100 g milk fat; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *iso* C16:0, C17:0 + C17:1 *cis*-9, C17:1 *cis*-9/C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *trans*-11,*cis* 15). Data of all measuring days except for d 7 of wk 6 were included in the PCA.

Because interanimal variability is most often observed, changes relative to standard conditions calculated on an individual animal basis could eliminate this interanimal variability and this has been done for milk *iso* C14:0 and C18:1 *trans*-10. Individual relative cow changes were calculated for this purpose as the difference (diff C18 *trans*-10, diff *iso* C14) and the ratio (ratio C18 *trans*-10, ratio *iso* C14) of the measurement of each of wk 2 to 6 with wk 1 (reference measurement).

A discriminant analysis (**DA**) was performed with SPSS (SPSS Inc.) using a stepwise model including the variables milk fat percentage, milk FA (g/100 g of milk fat; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *iso* C16:0, C17:0 + C17:1 *cis*-9, C17:1 *cis*-9/C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, C18:2 *trans*-11,*cis*-15), and the computed variables difference C18 *trans*-10, difference *iso* C14, ratio C18 *trans*-10, and ratio *iso* C14. Data of all measuring days were included in the PCA. For the purpose of the DA, acidotic and nonacidotic cases were distinguished according to the rumen pH using the threshold value (time pH lower than 5.6 ≥ 283 min) obtained by AlZahal et al. (2007). All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL).

## RESULTS

### Treatment Groups

The majority of the parameters considered in this study (rumen pH variables, rumen VFA concentration,

rumen VFA composition, DMI, water intake, milk yield, and milk composition including most of the milk FA) were not influenced by treatment group (Tables 4 to 8). Strikingly, acidotic indicators (i.e., rumen pH variables) did not differ between the groups, although supplements in the treatment group aimed at reducing the risk of acidosis development. Also, the number of cows qualitatively identified as acidotic (based on feed intake or fecal consistency) did not differ between the 2 treatment groups. Some parameters showed differences: based on wk 1 to 5, milk FA proportions of palmitic acid (C16:0) were higher in treatment A than in treatment B (36.8 ± 0.6 and 34.8 ± 0.6 g/100 g of FAME, respectively), and milk FA proportions of C18:1 *cis*-15 were lower in treatment A than in treatment B (0.054 ± 0.004 and 0.070 ± 0.004 g/100 g of FAME, respectively). Because these FA do not play a discriminative role to distinguish nonacidotic from acidotic cases, the treatment effect will not be emphasized further in this paper.

### Cow Performance, Milk Protein, and Milk Fat Content

Cow performance, milk protein, and milk fat content are presented in Table 4. The increased DMI on d 2 of wk 6 was the result of higher amounts of concentrates offered, according to the acidosis induction protocol (see earlier). However, after d 2, several cows were observed to refuse the concentrate, which was the main reason for ceasing the acidosis induction. The wheat-based concentrate was immediately replaced with the standard pelleted concentrate and DMI fully recovered after 1 to 2 d. Day 7 values are thus not representative of effects of SARA induction and are not included in Table 4.

Milk yield decreased from wk 1 to 5 for both d 2 and 7. Water uptake was higher on d 2 of wk 5 compared with wk 1, 3, and 4. Milk fat content was lower on d 2 and 7 of wk 5 compared with wk 2 and 4. Milk protein content was higher on d 2 of wk 5 compared with wk 1 to 4. On d 7, wk 4 and 5 had higher milk protein contents than wk 1 and 2.

Over the first 5 wk, a difference in fat content was observed between d 2 and 7. This difference could not be found between d 2 and 7 of wk 6, but differences in milk yield and water intake were observed.

### Rumen pH and VFA Parameters

Figure 1 shows pH curves based on d 2 of wk 1, 5, and 6 for an acidotic and nonacidotic cow as an example. These curves show normal diurnal pH curves, with the acidotic cow constantly showing lower pH values compared with the nonacidotic cow, even in the first week of the experiment. The average amount of lactate was 0.72 ± 0.17 μmol/mL based on the 6 wk.

Rumen variables are listed in Table 5. A weekly increase of the wheat-based concentrate consistently (both on d 2 and 7) negatively affected rumen minimum and average pH, acetate proportions, and total VFA concentrations, with the lowest acetate proportions observed in wk 5. Further, for some variables, week differences were observed on d 2 only (i.e., time pH <5.6, AUC pH <5.6, AUC pH <6.0, acetate and propionate proportions). A decrease of the acetate proportion and an increase of the propionate proportion on d 2 can be observed from wk 3 until wk 5. Butyrate proportions of d 2 are more fluctuating over the weeks, with the highest values at wk 4 and 5. Although the other parameters varied with week, the most extreme observations were not always in wk 5.

In wk 2, some extreme observations for rumen parameters were found. Both d 2 and 7 showed a lower average and minimum pH, a longer time pH <5.6, and a greater AUC pH <6.0 in wk 2 compared with wk 1, 3, 4, and 5.

On d 2, butyrate proportions and pH decrease per hour of wk 6 were different from wk 5. Rumen pH parameters did respond to the removal of wheat-based concentrate on d 7 in wk 6, but VFA proportions did not. No significant differences between d 2 and 7 were observed in wk 1 to 5 except for the total amount of VFA (mmol/L), which is also different between d 2 and 7 of wk 6. The latter is expected because of the removal of the wheat-based concentrate. A significant week effect is observed only 3 times on d 7 compared with 7 times on d 2.

Figure 2 shows the effect of the starch intake on the amount of time pH <5.6. Time pH <5.6 was highest for cows consuming the largest amounts of concentrates (pairs 1 and 2).

### Milk FA

Milk FA proportions are listed in Table 6 (short- and medium-chain FA), Table 7 (C18 FA), and Table 8 (OBCFA). Some milk FA changes (C16:0 and C18:0) are the result of the removal of the palm-based rumen-inert fat when wheat-based concentrate replaced the standard pelleted concentrate in the diet (Table 2). Unsaturated C18 FA decreased over time (d 2 results) except for C18:1 *trans*-10 and C18:1 *cis*-11 proportions, where a sudden increase in wk 5 was observed in contrast to a steady state (C18:1 *trans*-10) or gradual decrease (C18:1 *cis*-11) in the first 4 wk. On d 7, a sudden increase of most monounsaturated C18 FA (except for C18:1 *cis*-13, C18:1 *cis*-14 + C18:1 *trans*-15, and C18:1 *cis*-15) was observed in wk 5, mostly after a gradual

**Table 4.** Effect of gradual replacement of a standard dairy concentrate (sugar beet pulp/corn-based) with a wheat-based concentrate in a corn silage/grass silage/concentrate diet (33:17:50 wt/wt/wt; induction wk 1 to 5) and increase of the wheat-based concentrate (induction wk 6) on cow performance variables on d 2 and 7 of each induction week (n = 12)

| | | Induction week | | | | | | *P*-value (wk 1–5)[1] | | | Induction week | | *P*-value (wk 5–6)[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mean | 1 | 2 | 3 | 4 | 5 | SE (wk 1–5) | Week | Trt[2] | Cow | 6 | SE (wk 5–6) | Week | Trt[2] | Cow |
| Day 2 | | | | | | | | | | | 33.4 | | | | |
| Milk yield (kg/d) | 34.3 | 35.7$^{b}$ | 36.0$^{b}$ | 35.9$^{b}$ | 29.9$^{a}$ | 34.1$^{ab}$ | 4.17 | * | 0.799 | * | | 4.25 | 0.335 | 0.894 | * |
| DMI (kg/d) | 24.0 | 24.0 | 24.3 | 24.1 | 23.6 | 23.9 | 0.93 | 0.921 | 0.919 | * | 26.0 | 0.88 | ** | 0.965 | * |
| Water[3] (L/d) | 103 | 99$^{ab}$ | 105$^{bc}$ | 101$^{ab}$ | 97$^{a}$ | 111$^{c}$ | 6.5 | ** | 0.706 | * | 104 | 7.4 | † | 0.974 | * |
| Fat (g/100 g) | 4.58 | 4.56$^{ab}$ | 4.69$^{b}$ | —[4] | 4.68$^{b}$ | 4.37$^{a}$ | 0.239 | † | 0.423 | * | 4.18 | 0.279 | 0.147 | 0.936 | * |
| Protein (g/100 g) | 3.68 | 3.63$^{a}$ | 3.60$^{a}$ | —[4] | 3.68$^{a}$ | 3.81$^{b}$ | 0.142 | ** | 0.728 | * | 3.86 | 0.131 | 0.287 | 0.609 | * |
| Day 7 | | | | | | | | | | | | | | | |
| Milk yield (kg/d) | 33.5 | 37.1$^{c}$ | 34.7$^{bc}$ | 32.3$^{ab}$ | 32.8$^{ab}$ | 30.9$^{a}$ | 4.09 | * | 0.721 | * | 25.7[5] | 3.94 | * | 0.782 | * |
| DMI (kg/d) | 24.4 | 24.8 | 24.6 | 23.3 | 24.6 | 24.7 | 0.94 | 0.343 | 0.208 | * | — | — | — | — | — |
| Water[3] (L/d) | 102 | 101 | 101 | 97 | 109 | 103 | 7.5 | 0.565 | 0.249 | † | 91[5] | 6.9 | † | 0.769 | 0.103 |
| Fat (g/100 g) | 4.41[5] | 4.52$^{b}$ | 4.53$^{b}$ | 4.38$^{ab}$ | 4.40$^{ab}$ | 4.22$^{a}$ | 0.238 | 0.143 | 0.490 | * | 4.48 | 0.277 | 0.201 | 0.336 | † |
| Protein (g/100 g) | 3.71 | 3.61$^{a}$ | 3.62$^{a}$ | 3.72$^{ab}$ | 3.78$^{b}$ | 3.82$^{b}$ | 0.140 | ** | 0.626 | * | 3.86 | 0.131 | 0.287 | 0.609 | * |

[a–c]Means within a row in induction wk 1 to 5 with different superscripts differ ($P < 0.05$).

[1]*P*-values according to the linear mixed model for wk 1 to 5 and for wk 5 to 6. Analysis performed with SPSS 15.0.0 (SPSS Inc., Chicago, IL). $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term.

[2]Trt = treatment.

[3]In wk 4, water supply of 1 cow was broken.

[4]Samples for analysis of fat and protein of wk 3 d 2 were lost.

[5]Significant difference between d 2 and 7 either over induction wk 1 to 5 (mean) or in wk 6 ($P < 0.05$) according to the linear mixed models (SPSS 15.0.0). Induction wk 1 to 5: $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term. Week 6: $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; †$0.05 < P < 0.10$.

decrease in the first 4 wk. An exception to this general trend was C18:1 *trans*-11, which decreased from wk 1 to 5.

Odd- and branched-chain FA, which significantly changed with week, generally showed an increase, except for *anteiso* C15:0, which decreased, and *iso* C14:0 and *iso* C15:0, which decreased in wk 5 after their gradual increase during the first 4 wk. Increasing the amount of concentrate in wk 6 (d 2) further increased C17:0 and C15:0 and reduced *iso* C14:0 and *iso* C16:0 proportions compared with wk 5.

After returning to a standard ration between d 2 and 7 of wk 6, only *anteiso* C15:0 returned to a concentration similar to wk 1, whereas other OBCFA did not yet show a difference from wk 5 concentrations or even continued to increase (*anteiso* C13:0, C17:0, and, as a consequence, C17:0 + C17:1 *cis*-9).

### PCA

The first and second principal components (**PC1** and **PC2**, respectively; Figure 3a) described 39% of the to-

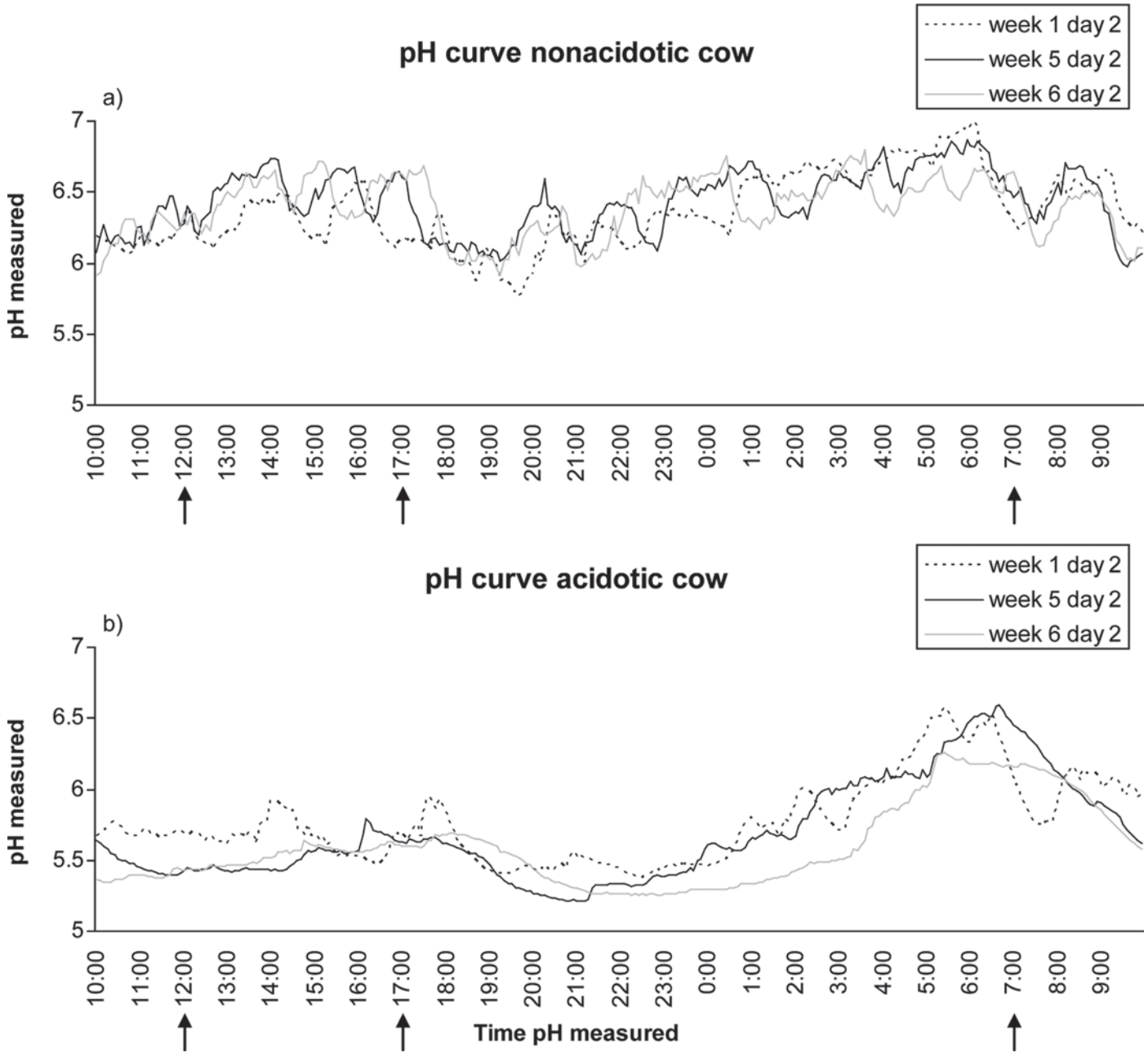


**Figure 1.** pH curves of (a) a nonacidotic cow and (b) an acidotic cow on d 2 (24 h) of wk 1, 5, and 6. Arrow indicates time of feeding.

**Table 5.** Effect of gradual replacement of a standard dairy concentrate (sugar beet pulp/corn-based) with a wheat-based concentrate in a corn silage/grass silage/concentrate diet (33:17:50 wt/wt/wt; induction wk 1 to 5) and increase of the wheat-based concentrate (induction wk 6) on rumen variables on d 2 and 7 of each induction week (n = 12)

| | | Induction week | | | | | | *P*-value (wk 1–5)[1] | | | Induction week | | *P*-value (wk 1–5)[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mean | 1 | 2 | 3 | 4 | 5 | SE (wk 1–5) | Week | Trt[2] | Cow | 6 | SE (wk 1–5) | Week | Trt[2] | Cow |
| Day 2 | | | | | | | | | | | | | | | |
| Average pH | 5.95 | 6.01$^{b}$ | 5.90$^{a}$ | 5.99$^{b}$ | 5.91$^{a}$ | 5.96$^{ab}$ | 0.066 | † | 0.719 | * | 5.92 | 0.085 | 0.487 | 0.743 | † |
| pH minimum | 5.47 | 5.52$^{bc}$ | 5.41$^{a}$ | 5.54$^{c}$ | 5.44$^{ab}$ | 5.45$^{ab}$ | 0.063 | * | 0.707 | * | 5.39 | 0.080 | 0.681 | 0.600 | † |
| pH maximum | 6.61 | 6.69 | 6.60 | 6.60 | 6.54 | 6.64 | 0.065 | 0.424 | 0.471 | 0.155 | 6.55 | 0.075 | 0.169 | 0.459 | † |
| pH decrease/h | 0.136 | 0.090$^{a}$ | 0.141$^{b}$ | 0.149$^{b}$ | 0.158$^{b}$ | 0.143$^{b}$ | 0.016 | † | 0.910 | 0.699 | 0.216 | 0.020 | * | 0.748 | 0.984 |
| Time pH <5.6 (min/d) | 275 | 157$^{a}$ | 363$^{b}$ | 215$^{a}$ | 282$^{ab}$ | 359$^{b}$ | 88.4 | * | 0.639 | * | 375 | 107 | 0.833 | 0.772 | † |
| Time pH <6.0 (min/d) | 813 | 747 | 883 | 775 | 886 | 775 | 105.3 | 0.221 | 0.738 | * | 800 | 127 | 0.823 | 0.558 | † |
| AUC[3] pH <5.6 (min × pH/d) | 37.3 | 11.0$^{a}$ | 49.1$^{bc}$ | 29.3$^{ab}$ | 38.9$^{bc}$ | 58.4$^{c}$ | 13.99 | * | 0.894 | † | 72.7 | 25.2 | 0.435 | 0.912 | † |
| AUC pH <6.0 (min × pH/d) | 249 | 152$^{a}$ | 298$^{b}$ | 234$^{b}$ | 271$^{b}$ | 290$^{b}$ | 51.6 | ** | 0.566 | * | 308 | 69.5 | 0.709 | 0.735 | † |
| Acetate (% mmol) | 61.1 | 62.1$^{c}$ | 61.5$^{bc}$ | 62.3$^{c}$ | 60.5$^{b}$ | 59.0$^{a}$ | 0.92 | * | 0.835 | * | 59.2 | 1.21 | 0.535 | 0.759 | *** |
| Propionate (% mmol) | 20.1 | 19.3$^{a}$ | 20.5$^{bc}$ | 19.4$^{ab}$ | 20.0$^{abc}$ | 21.2$^{c}$ | 0.77 | ** | 0.854 | * | 21.4 | 1.31 | 0.507 | 0.751 | ** |
| Butyrate (% mmol) | 14.4 | 14.0$^{ab}$ | 14.0$^{ab}$ | 13.6$^{a}$ | 14.8$^{bc}$ | 15.4$^{c}$ | 0.41 | ** | 0.930 | 0.169 | 14.1 | 0.54 | ** | 0.557 | † |
| Total VFA (mmol/L) | 108 | 117$^{b}$ | 113$^{ab}$ | 103$^{a}$ | 102$^{a}$ | 106$^{ab}$ | 4.9 | † | 0.721 | 0.122 | 94.6 | 3.87 | † | 0.322 | — |
| Day 7 | | | | | | | | | | | | | | | |
| Average pH | 5.94 | 5.87$^{a}$ | 6.01$^{b}$ | 5.95$^{ab}$ | 5.95$^{ab}$ | 5.92$^{ab}$ | 0.078 | * | 0.755 | * | 6.14[4] | 0.078 | ** | 0.807 | † |
| pH minimum | 5.47 | 5.42$^{a}$ | 5.53$^{b}$ | 5.52$^{b}$ | 5.52$^{b}$ | 5.37$^{a}$ | 0.076 | *** | 0.786 | * | 5.61[4] | 0.072 | ** | 0.843 | † |
| pH maximum | 6.59 | 6.54 | 6.59 | 6.60 | 6.60 | 6.60 | 0.061 | 0.710 | 0.251 | * | 6.65 | 0.064 | 0.180 | 0.827 | † |
| pH decrease/h | 0.148 | 0.134$^{ab}$ | 0.121$^{a}$ | 0.153$^{ab}$ | 0.151$^{ab}$ | 0.179$^{b}$ | 0.020 | 0.301 | 0.920 | *** | 0.111[4] | 0.144 | * | 0.902 | — |
| Time pH <5.6 (min/d) | 314 | 354 | 218 | 326 | 326 | 347 | 102.9 | 0.307 | 0.756 | * | 52[4] | 73.4 | ** | 0.764 | 0.541 |
| Time pH <6.0 (min/d) | 813 | 915$^{b}$ | 710$^{a}$ | 801$^{ab}$ | 801$^{ab}$ | 837$^{ab}$ | 122.8 | 0.153 | 0.586 | * | 568[4] | 124.5 | ** | 0.966 | † |
| AUC <5.6 (min × pH/d) | 66.1 | 61.5$^{ab}$ | 22.7$^{a}$ | 127.5$^{b}$ | 47.0$^{ab}$ | 71.8$^{ab}$ | 22.93 | 0.326 | 0.514 | 0.155 | 3.97 | 20.89 | * | 0.783 | 0.891 |
| AUC <6.0 (min × pH/d) | 299 | 322$^{ab}$ | 214$^{a}$ | 369$^{b}$ | 271$^{ab}$ | 318$^{ab}$ | 79.6 | 0.320 | 0.954 | † | 119 | 51.920 | * | 0.967 | — |
| Acetate (% mmol) | 61.3 | 63.0$^{c}$ | 62.4$^{b}$ | 61.2$^{ab}$ | 60.3$^{a}$ | 59.5$^{a}$ | 0.88 | *** | 0.584 | † | 59.1 | 1.18 | 0.624 | 0.881 | * |
| Propionate (% mmol) | 20.1 | 19.8$^{ab}$ | 19.1$^{a}$ | 19.6$^{ab}$ | 21.2$^{b}$ | 20.9$^{ab}$ | 0.90 | 0.142 | 0.512 | † | 21.3 | 1.48 | 0.584 | 0.881 | *** |
| Butyrate (% mmol) | 14.3 | 13.5$^{a}$ | 14.0$^{ab}$ | 14.9$^{b}$ | 14.2$^{ab}$ | 14.9$^{b}$ | 0.42 | † | 0.301 | — | 14.1 | 0.61 | 0.206 | 0.834 | 0.100 |
| Total VFA (mmol/L) | 118[4] | 120$^{ab}$ | 110$^{a}$ | 126$^{b}$ | 122$^{ab}$ | 114$^{ab}$ | 5.1 | † | 0.906 | 0.135 | 103[4] | 4.7 | * | 0.805 | † |

[a–c]Means within a row in induction wk 1 to 5 with different superscripts differ ($P < 0.05$).

[1]*P*-values according to the linear mixed model for wk 1 to 5 and for wk 5 to 6. Analysis performed with SPSS 15.0.0 (SPSS Inc., Chicago, IL). $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term.

[2]Trt = treatment.

[3]AUC = area under the curve.

[4]Significant difference between d 2 and 7 either over induction wk 1 to 5 (mean) or in wk 6 ($P < 0.05$) according to the linear mixed models (SPSS 15.0.0). Induction wk 1 to 5: $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term. Week 6: $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$; †$0.05 < P < 0.10$.

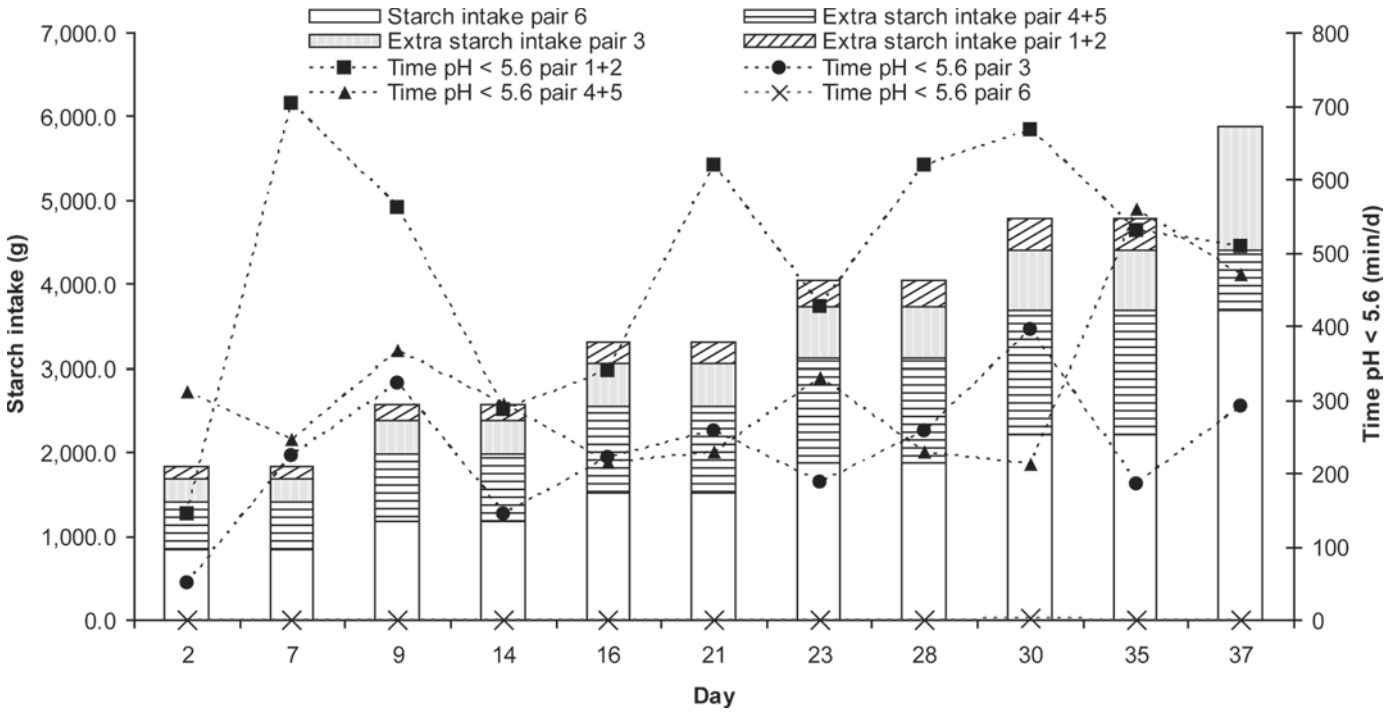


**Figure 2.** Starch intake (g) and time pH <5.6 (min/d) for pairs 1 + 2, 3, 4 + 5, and 6. Blank bar = starch intake of pair 6; horizontally shaded bars = extra amount of starch intake by pairs 4 and 5 with respect to pair 6; vertically shaded bars = extra amount of starch intake by pair 3 with respect to pairs 4 and 5; diagonally shaded bars = extra amount of starch intake by pairs 1 and 2 with respect to pair 3.

tal variation in rumen pH variables, rumen VFA, milk FA proportions of C18 biohydrogenation intermediates (C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *trans*-10,*cis*-12, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-11,*cis*-15), and OBCFA. Milk *iso* FA proportions (*iso* C14:0, *iso* C15:0, and, to a lesser extent, *iso* C13:0 and *iso* C16:0), rumen acetate proportions, and rumen minimal pH were positively correlated to PC1. Milk fat content had scores of 0.268 and −0.047 for PC1 and PC2, respectively (Figure 3a), suggesting milk fat percentage to be less correlated with pH variables (time pH <5.6 or 6.0 and pH minimum) and acetate proportions than *iso* C14:0 and *iso* C15:0. Time pH <5.6 and time pH <6.0 clustered to some extent with milk C15:0 on the opposite side of PC1. Proportions of C17:0 + C17:1 *cis*-9 and pH decrease per hour showed a negative loading for both PC1 and PC2. Milk C18:1 *trans*-10 proportions clustered with rumen propionate proportions, showing a negative loading for PC1 and a positive loading for PC2. C18:2 *cis*-9,*trans*-11, C18:2 *trans*-11,*cis*-15, and C18:1 *trans*-11 showed the highest positive loadings on PC2, whereas *anteiso* C13:0 had the greatest negative loading.

Score plots (Figure 3b and 3c) showed that cow scores were mainly distributed along the PC1 axis, whereas the scores of the different weeks were mainly distributed along the PC2 axis, particularly for wk 1 to 4. Standard errors of the mean of PC1 increased with induction week (from 0.13 in wk 1 to 0.40 in wk 6; Figure 3c). Moreover, cows showing clinical signs of acidosis based on the fecal scoring (cows 1, 2, 5, 6, 7, 8, and 12 in Figure 3b) also showed higher changes in *iso* C14:0 and C18:1 *trans*-10 concentrations in milk fat over the weeks. Calculation of the coefficient of variance of C18:1 *trans*-10 per week showed an increase from 0.18 in wk 1 to 0.75 in wk 6. This was also seen on the score plot of the different cows, where cows 5 and 6, identified as suffering from acidosis, had larger standard errors for PC1 than cows 3 and 4, which were identified as healthy cows.

### *DA*

To discriminate between acidotic and nonacidotic cows (defined by rumen pH according to AlZahal et al., 2007), a stepwise DA was performed based on milk FA, milk fat percentage, and the relative changes of C18:1 *trans*-10 and *iso* C14:0. The most discriminating variables were C18:2 *cis*-9,*trans*-11, *iso* C16:0, and *iso* C13:0, with standardized canonical discriminant function coefficients 0.866, 0.687, and 0.505, respectively. The fraction of correctly classified cases using cross-validation was 65.2% of 138 cases (Table 9). Acidotic cases that were falsely classified as control showed lower discrimi-

**Table 6.** Effect of gradual replacement of a standard dairy concentrate (sugar beet pulp/corn-based) with a wheat-based concentrate in a corn silage/grass silage/concentrate diet (33:17:50 wt/wt/wt; induction wk 1 to 5) and increase of the wheat-based concentrate (induction wk 6) on milk short- and medium-chain fatty acids proportions (wt %) on d 2 and 7 of each induction week (n = 12)

| | | Induction week | | | | | SE (wk 1–5) | *P*-value (wk 1–5)[1] | | | Induction week | SE (wk 5–6) | *P*-value (wk 5–6)[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mean | 1 | 2 | 3 | 4 | 5 | | Week | Trt[2] | Cow | 6 | | Week | Trt[2] | Cow |
| Day 2 | | | | | | | | | | | | | | | |
| C4:0 | 4.59 | 4.54 | 4.49 | 4.64 | 4.72 | 4.56 | 0.148 | 0.340 | 0.712 | * | 4.47 | 0.156 | 0.240 | 0.702 | * |
| C6:0 | 2.44 | 2.33$^{a}$ | 2.36$^{a}$ | 2.72$^{b}$ | 2.40$^{a}$ | 2.39$^{a}$ | 0.080 | *** | 0.996 | * | 2.53 | 0.085 | ** | 0.716 | * |
| C8:0 | 1.29 | 1.13$^{a}$ | 1.22$^{b}$ | 1.43$^{d}$ | 1.33$^{c}$ | 1.36$^{c}$ | 0.043 | *** | 0.633 | * | 1.56 | 0.049 | *** | 0.424 | * |
| C10:0 | 2.91 | 3.19$^{b}$ | 3.04$^{b}$ | 3.21$^{b}$ | 2.55$^{a}$ | 2.56$^{a}$ | 0.125 | *** | 0.311 | * | 2.86 | 0.103 | *** | 0.359 | * |
| C12:0 | 3.70 | 3.20$^{a}$ | 3.30$^{ab}$ | 3.80$^{b}$ | 4.11$^{c}$ | 4.41$^{d}$ | 0.146 | *** | 0.217 | * | 4.84 | 0.154 | ** | 0.161 | † |
| C14:0 | 11.6 | 10.9$^{a}$ | 11.0$^{a}$ | 11.4$^{b}$ | 12.3$^{c}$ | 12.6$^{c}$ | 0.28 | *** | 0.239 | * | 12.9 | 0.30 | *** | 0.101 | * |
| C14:1 *cis*-9 | 1.26 | 1.14$^{a}$ | 1.23$^{b}$ | 1.24$^{b}$ | 1.29$^{b}$ | 1.38$^{c}$ | 0.121 | *** | 0.802 | * | 1.43 | 0.128 | † | 0.656 | * |
| C16:0 | 37.0 | 37.9$^{c}$ | 37.4$^{c}$ | 37.4$^{c}$ | 36.6$^{b}$ | 35.6$^{a}$ | 0.50 | *** | * | * | 34.7 | 0.52 | * | * | * |
| C16:1 *cis*-9 | 2.44 | 2.33$^{a}$ | 2.56$^{c}$ | 2.48$^{bc}$ | 2.41$^{ab}$ | 2.43$^{ac}$ | 0.158 | * | 0.491 | * | 2.53 | 0.144 | † | 0.136 | * |
| Day 7 | | | | | | | | | | | | | | | |
| C4:0 | 4.57 | 4.60 | 4.59 | 4.63 | 4.62 | 4.43 | 0.137 | 0.283 | 0.861 | * | 4.10[3] | 0.157 | ** | 0.953 | * |
| C6:0 | 2.50[3] | 2.31$^{a}$ | 2.45$^{b}$ | 2.48$^{b}$ | 2.62$^{c}$ | 2.61$^{c}$ | 0.080 | *** | 0.618 | * | 2.47 | 0.10 | ** | 0.508 | * |
| C8:0 | 1.72[3] | 1.92$^{a}$ | 1.69$^{b}$ | 2.16$^{c}$ | 1.38$^{d}$ | 1.43$^{d}$ | 0.089 | *** | 0.745 | † | 1.42 | 0.050 | 0.773 | 0.136 | * |
| C10:0 | 3.52[3] | 3.77$^{a}$ | 3.72$^{a}$ | 3.99$^{a}$ | 3.09$^{b}$ | 3.01$^{b}$ | 0.179 | *** | 0.129 | † | 3.32[3] | 0.154 | † | * | 0.146 |
| C12:0 | 3.72 | 3.05$^{a}$ | 3.42$^{b}$ | 3.66$^{c}$ | 4.03$^{d}$ | 4.46$^{e}$ | 0.149 | *** | 0.143 | * | 4.71 | 0.154 | 0.111 | * | 0.112 |
| C14:0 | 11.5[3] | 10.4$^{a}$ | 11.1$^{b}$ | 11.5$^{c}$ | 12.0$^{d}$ | 12.3$^{d}$ | 0.26 | *** | 0.11 | * | 12.3[3] | 0.30 | † | 0.830 | * |
| C14:1 *cis*-9 | 1.31[3] | 1.19$^{a}$ | 1.21$^{a}$ | 1.25$^{a}$ | 1.42$^{b}$ | 1.47$^{b}$ | 0.123 | *** | 0.727 | * | 1.53 | 0.121 | 0.494 | 0.354 | † |
| C16:0 | 35.8[3] | 36.6$^{c}$ | 36.6$^{c}$ | 35.8$^{b}$ | 35.9$^{bc}$ | 34.3$^{a}$ | 0.50 | *** | * | * | 32.8[3] | 0.58 | * | † | † |
| C16:1 *cis*-9 | 2.22[3] | 2.23 | 2.12 | 2.11 | 2.31 | 2.32 | 0.164 | 0.152 | 0.286 | * | 2.36[3] | 0.146 | 0.689 | * | * |

[a–e]Means within a row in induction wk 1 to 5 with different superscripts differ ($P < 0.05$).

[1]*P*-values according to the linear mixed model for wk 1 to 5 and for wk 5 to 6. Analysis performed with SPSS 15.0.0 (SPSS Inc., Chicago, IL). $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term.

[2]Trt = treatment.

[3]Significant difference between d 2 and 7 either over induction wk 1 to 5 (mean) or in wk 6 ($P < 0.05$) according to the linear mixed models (SPSS 15.0.0). Induction wk 1 to 5: $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term. Week 6: $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$; †$0.05 < P < 0.10$.

nant scores (i.e., closer to the discriminant scores of the acidotic cases (Figure 4). However, discriminant scores of control cases falsely classified as acidotic showed a similar range as observed for the correctly classified acidotic cases. This could suggest that the discriminant model is more likely to misclassify control cases as acidotic rather than acidotic cases as control.

## DISCUSSION

Almost no differences between both treatment groups, and no interactions between treatment and induction week, were recorded except for 2 FA. Therefore, this discussion focuses on effects of induction week across both treatment groups.

### *Rumen pH Changes During Induction Protocol*

Based on the threshold value for subacute acidosis (time pH below 5.6 $\geq$ 283 min/d) as proposed by AlZahal et al. (2007), the herd could be classified on average as acidotic on d 2 of wk 2, 5, and 6 (8, 5, and 7 cows classified as acidotic, respectively). This means that the SARA induction protocol was successful. Although the lowest minimum pH and the highest time pH <5.6 were observed in wk 2, cows seemed to recover until wk 5. Hence, cows may suffer from subacute acidosis during a short period (wk 2). This is in accordance with a greater number of significant week effects on d 2 compared with d 7 for rumen pH and VFA parameters, suggesting that cows were able to adapt during the week following a dietary shift. This is also reported by Sun et al. (2010). In wk 5 and 6 some cows showed clinical signs of acidosis as based on refusal of DMI and fecal consistency.

### *Milk OBCFA and Their Relation with Rumen Characteristics*

A positive correlation between the acetate proportion and *iso* C14:0 and *iso* C15:0 concentrations in milk fat and between C15:0, C17:0, and propionate proportions

**Table 7.** Effect of gradual replacement of a standard dairy concentrate (sugar beet pulp/corn-based) with a wheat-based concentrate in a corn silage/grass silage/concentrate diet (33:17:50 wt/wt/wt; induction wk 1 to 5) and increase of the wheat-based concentrate (induction wk 6) on milk C18 saturated and unsaturated fatty acids proportions (wt %) on d 2 and 7 of each induction week (n = 12)

| | | Induction week | | | | | SE (wk | *P*-value (wk 1–5)[1] | | | Induction week | SE (wk | *P*-value (wk 5–6)[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mean | 1 | 2 | 3 | 4 | 5 | 1–5) | Week | Trt[2] | Cow | 6 | 5–6) | Week | Trt[2] | Cow |
| Day 2 | | | | | | | | | | | | | | | |
| C18:0 | 7.15 | 8.05$^{b}$ | 7.81$^{b}$ | 6.46$^{a}$ | 6.71$^{a}$ | 6.71$^{a}$ | 0.276 | *** | 0.621 | 0.769 | 6.86 | 0.209 | 0.466 | 0.670 | 0.124 |
| C18:1 *trans*-6 + C18:1 *trans*-8 | 0.177 | 0.185$^{c}$ | 0.183$^{bc}$ | 0.178$^{b}$ | 0.169$^{a}$ | 0.171$^{ab}$ | 0.007 | *** | 0.199 | * | 0.172 | 0.008 | 0.776 | 0.429 | * |
| C18:1 *trans*-9 | 0.157 | 0.165$^{c}$ | 0.163$^{c}$ | 0.158$^{bc}$ | 0.148$^{a}$ | 0.152$^{ab}$ | 0.004 | *** | 0.115 | * | 0.149 | 0.006 | 0.333 | 0.843 | * |
| C18:1 *cis*-9 | 16.3 | 16.3 | 16.3 | 16.3 | 16.2 | 16.3 | 0.48 | 0.962 | 0.295 | * | 15.4 | 0.57 | ** | 0.373 | * |
| C18:1 *trans*-10 | 0.247 | 0.242$^{a}$ | 0.241$^{a}$ | 0.240$^{a}$ | 0.237$^{a}$ | 0.275$^{b}$ | 0.016 | * | 0.330 | * | 0.346 | 0.040 | * | 0.993 | * |
| C18:1 *trans*-11 | 0.718 | 0.786$^{c}$ | 0.774$^{c}$ | 0.713$^{b}$ | 0.665$^{ab}$ | 0.649$^{a}$ | 0.044 | *** | 0.996 | * | 0.544 | 0.040 | ** | 0.812 | * |
| C18:1 *cis*-11 | 0.497 | 0.494$^{a}$ | 0.485$^{a}$ | 0.475$^{a}$ | 0.469$^{a}$ | 0.563$^{b}$ | 0.027 | ** | 0.442 | * | 0.501 | 0.033 | ** | 0.898 | * |
| C18:1 *trans*-12 | 0.391 | 0.408$^{a}$ | 0.407$^{a}$ | 0.397$^{a}$ | 0.363$^{b}$ | 0.383$^{ab}$ | 0.012 | * | 0.186 | 0.201 | 0.342 | 0.013 | *** | 0.776 | * |
| C18:1 *cis*-12 | 0.253 | 0.257 | 0.254 | 0.248 | 0.247 | 0.257 | 0.012 | 0.529 | 0.245 | * | 0.245 | 0.013 | 0.198 | 0.633 | † |
| C18:1 *cis*-13 | 0.036 | 0.030$^{a}$ | 0.041$^{b}$ | 0.036$^{ab}$ | 0.034$^{ab}$ | 0.041$^{b}$ | 0.008 | † | 0.331 | * | 0.047 | 0.009 | † | 0.641 | * |
| C18:1 *cis*-14 + C18:1 *trans*-15 | 0.284 | 0.275$^{a}$ | 0.285$^{ab}$ | 0.291$^{b}$ | 0.283$^{ab}$ | 0.287$^{ab}$ | 0.010 | 0.269 | 0.160 | * | 0.265 | 0.012 | * | 0.370 | * |
| C18:1 *cis*-15 | 0.062 | 0.063$^{ab}$ | 0.068$^{a}$ | 0.068$^{a}$ | 0.060$^{ab}$ | 0.052$^{b}$ | 0.003 | * | * | 0.151 | 0.054 | 0.006 | 0.740 | 0.470 | † |
| C18:2 *trans trans* | 0.341 | 0.319$^{a}$ | 0.337$^{ab}$ | 0.330$^{ab}$ | 0.347$^{bc}$ | 0.372$^{c}$ | 0.012 | ** | † | 0.11 | 0.340 | 0.019 | 0.209 | 0.271 | 0.554 |
| C18:2 *cis*-9,*trans*-11 | 0.417 | 0.428$^{ac}$ | 0.460$^{a}$ | 0.421$^{bc}$ | 0.388$^{b}$ | 0.390$^{b}$ | 0.018 | ** | 0.272 | † | 0.336 | 0.021 | ** | 0.344 | * |
| C18:2 *trans*-10,*cis*-12 | 0.012 | 0.010$^{a}$ | 0.011$^{ac}$ | 0.011$^{ac}$ | 0.013$^{bc}$ | 0.015$^{b}$ | 0.001 | ** | 0.916 | 0.381 | 0.016 | 0.001 | 0.718 | 0.612 | 0.988 |
| C18:2 *trans*-11,*cis*-15 | 0.060 | 0.053$^{a}$ | 0.065$^{b}$ | 0.064$^{bc}$ | 0.058$^{ac}$ | 0.061$^{bc}$ | 0.004 | ** | 0.650 | † | 0.051 | 0.004 | † | 0.658 | 0.98 |
| C18:2 n-6 | 1.44 | 1.36$^{a}$ | 1.40$^{a}$ | 1.39$^{a}$ | 1.54$^{b}$ | 1.52$^{b}$ | 0.075 | *** | 0.354 | * | 1.64 | 0.059 | * | 0.485 | † |
| C18:3 n-6 | 0.026 | 0.026 | 0.026 | 0.026 | 0.028 | 0.025 | 0.002 | 0.423 | 0.371 | 0.101 | 0.028 | 0.002 | 0.192 | 0.752 | 0.364 |
| C18:3 n-3 | 0.352 | 0.320$^{a}$ | 0.350$^{b}$ | 0.341$^{b}$ | 0.369$^{c}$ | 0.381$^{c}$ | 0.013 | *** | 0.668 | * | 0.414 | 0.010 | * | 0.907 | 0.275 |
| Day 7 | | | | | | | | | | | | | | | |
| C18:0 | 7.08 | 7.99$^{a}$ | 7.85$^{a}$ | 6.21$^{b}$ | 6.45$^{b}$ | 6.92$^{b}$ | 0.306 | *** | 0.824 | 0.984 | 7.01 | 0.295 | 0.768 | 0.793 | 0.130 |
| C18:1 *trans*-6 + C18:1 *trans*-8 | 0.200[3] | 0.213$^{a}$ | 0.202$^{b}$ | 0.198$^{b}$ | 0.186$^{c}$ | 0.203$^{b}$ | 0.007 | *** | 0.216 | * | 0.215[3] | 0.014 | 0.463 | 0.35 | 0.335 |
| C18:1 *trans*-9 | 0.162[3] | 0.171$^{a}$ | 0.163$^{b}$ | 0.161$^{b}$ | 0.149$^{c}$ | 0.163$^{b}$ | 0.004 | *** | 0.198 | * | 0.175 | 0.010 | 0.301 | 0.613 | 0.247 |
| C18:1 *cis*-9 | 16.4 | 16.5 | 15.9 | 16.7 | 16.4 | 16.4 | 0.50 | * | 0.260 | * | 16.7[3] | 0.51 | 0.551 | 0.281 | 0.108 |
| C18:1 *trans*-10 | 0.288[3] | 0.263$^{a}$ | 0.261$^{a}$ | 0.262$^{a}$ | 0.276$^{a}$ | 0.375$^{b}$ | 0.025 | *** | 0.515 | * | 0.614[3] | 0.101 | * | 0.92 | 0.162 |
| C18:1 *trans*-11 | 0.702 | 0.829$^{a}$ | 0.763$^{b}$ | 0.709$^{c}$ | 0.621$^{d}$ | 0.588$^{d}$ | 0.042 | *** | 0.952 | * | 0.652 | 0.051 | 0.384 | 0.853 | 0.910 |
| C18:1 *cis*-11 | 0.564[3] | 0.504$^{a}$ | 0.514$^{a}$ | 0.563$^{b}$ | 0.579$^{b}$ | 0.661$^{c}$ | 0.027 | *** | 0.323 | * | 0.694[3] | 0.042 | 0.170 | 0.768 | * |
| C18:1 *trans*-12 | 0.351[3] | 0.373$^{c}$ | 0.355$^{bc}$ | 0.347$^{ab}$ | 0.329$^{a}$ | 0.349$^{abc}$ | 0.012 | * | 0.354 | † | 0.328 | 0.018 | 0.407 | 0.211 | 0.974 |
| C18:1 *cis*-12 | 0.262[3] | 0.260$^{ab}$ | 0.256$^{ab}$ | 0.264$^{a}$ | 0.251$^{b}$ | 0.280$^{c}$ | 0.012 | *** | 0.614 | * | 0.272 | 0.018 | 0.724 | 0.248 | 0.653 |
| C18:1 *cis*-13 | 0.052[3] | 0.051 | 0.052 | 0.052 | 0.054 | 0.051 | 0.006 | 0.965 | 0.467 | * | 0.064[3] | 0.007 | † | 0.966 | † |
| C18:1 *cis*-14 + C18:1 *trans*-15 | 0.312[3] | 0.331 | 0.308 | 0.316 | 0.309 | 0.296 | 0.020 | 0.533 | 0.891 | † | 0.280 | 0.020 | 0.389 | 0.992 | 0.104 |
| C18:1 *cis*-15 | 0.087[3] | 0.090 | 0.081 | 0.086 | 0.090 | 0.088 | 0.005 | 0.435 | † | 0.164 | 0.086[3] | 0.005 | 0.802 | 0.879 | 0.918 |
| C18:2 *trans trans* | 0.210[3] | 0.192$^{a}$ | 0.201$^{a}$ | 0.200$^{a}$ | 0.216$^{ab}$ | 0.239$^{b}$ | 0.014 | * | 0.855 | † | 0.237[3] | 0.019 | 0.907 | 0.719 | 0.361 |
| C18:2 *cis*-9,*trans*-11 | 0.472[3] | 0.527$^{a}$ | 0.489$^{b}$ | 0.467$^{bd}$ | 0.445$^{cd}$ | 0.433$^{c}$ | 0.019 | *** | 0.308 | * | 0.475[3] | 0.040 | 0.398 | 0.516 | 0.356 |
| C18:2 *trans*-10,*cis*-12 | 0.015[3] | 0.015 | 0.016 | 0.015 | 0.015 | 0.014 | 0.002 | 0.974 | 0.823 | 0.791 | 0.015 | 0.002 | 0.755 | 0.789 | 0.242 |
| C18:2 *trans*-11,*cis*-15 | 0.068[3] | 0.079$^{a}$ | 0.073$^{ab}$ | 0.062$^{cd}$ | 0.068$^{bc}$ | 0.058$^{d}$ | 0.004 | *** | 0.329 | † | 0.067[3] | 0.007 | 0.320 | 0.713 | 0.782 |
| C18:2 n-6 | 1.44 | 1.34$^{a}$ | 1.37$^{a}$ | 1.43$^{b}$ | 1.45$^{b}$ | 1.61$^{c}$ | 0.067 | *** | 0.324 | * | 1.82[3] | 0.081 | * | 0.321 | † |
| C18:3 n-6 | 0.068[3] | 0.031$^{a}$ | 0.069$^{b}$ | 0.032$^{a}$ | 0.106$^{c}$ | 0.103$^{c}$ | 0.007 | *** | 0.128 | 0.338 | 0.106[3] | 0.007 | 0.403 | 0.236 | * |
| C18:3 n-3 | 0.111[3] | 0.112$^{ab}$ | 0.106$^{a}$ | 0.117$^{b}$ | 0.113$^{b}$ | 0.105$^{a}$ | 0.005 | ** | 0.743 | * | 0.105[3] | 0.005 | 0.998 | 0.621 | † |

$^{a–d}$Means within a row in induction wk 1 to 5 with different superscripts differ ($P < 0.05$).

[1]*P*-values according to the linear mixed model for wk 1 to 5 and for wk 5 to 6. Analysis performed with SPSS 15.0.0 (SPSS Inc., Chicago, IL). $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term.

[2]Trt = treatment.

[3]Significant difference between d 2 and 7 either over induction wk 1 to 5 (mean) or in wk 6 ($P < 0.05$) according to the linear mixed models (SPSS 15.0.0). Induction wk 1 to 5: $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term. Week 6: $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$; †$0.05 < P < 0.10$.

**Table 8.** Effect of gradual replacement of a standard dairy concentrate (sugar beet pulp/corn-based) with a wheat-based concentrate in a corn silage/grass silage/concentrate diet (33:17:50 wt/wt/wt; induction wk 1 to 5) and increase of the wheat-based concentrate (induction wk 6) on odd- and branched-chain fatty acid proportions (wt %) on d 2 and 7 of each induction week (n = 12)

| | | Induction week | | | | | SE (wk 1–5) | *P*-value (wk 1–5)[1] | | | Induction week | SE (wk 5–6) | *P*-value (wk 5–6)[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Mean | 1 | 2 | 3 | 4 | 5 | | Week | Trt[2] | Cow | 6 | | Week | Trt[2] | Cow |
| Day 2 | | | | | | | | | | | | | | | |
| *iso* C13:0 | 0.026 | 0.024$^{ab}$ | 0.023$^{a}$ | 0.026$^{b}$ | 0.028$^{c}$ | 0.028$^{c}$ | 0.001 | *** | 0.391 | † | 0.029 | 0.002 | 0.287 | 0.629 | * |
| *anteiso* C13:0 | 0.113 | 0.097$^{a}$ | 0.105$^{b}$ | 0.108$^{b}$ | 0.121$^{c}$ | 0.135$^{d}$ | 0.007 | *** | 0.922 | * | 0.145 | 0.008 | * | 0.933 | * |
| *iso* C14:0 | 0.077 | 0.077$^{ab}$ | 0.076$^{ab}$ | 0.076$^{ab}$ | 0.081$^{b}$ | 0.073$^{a}$ | 0.005 | 0.150 | 0.812 | * | 0.065 | 0.005 | ** | 0.476 | * |
| C15:0 | 1.15 | 1.08$^{a}$ | 1.08$^{a}$ | 1.08$^{a}$ | 1.16$^{a}$ | 1.37$^{b}$ | 0.053 | *** | 0.736 | † | 1.52 | 0.113 | * | 0.457 | * |
| *iso* C15:0 | 0.206 | 0.203$^{a}$ | 0.202$^{a}$ | 0.207$^{ab}$ | 0.214$^{b}$ | 0.206$^{ab}$ | 0.006 | * | 0.520 | * | 0.203 | 0.006 | 0.450 | 0.415 | * |
| *anteiso* C15:0 | 0.445 | 0.468$^{d}$ | 0.457$^{cd}$ | 0.435$^{ab}$ | 0.442$^{bc}$ | 0.423$^{a}$ | 0.010 | *** | 0.807 | * | 0.437 | 0.010 | 0.220 | 0.616 | 0.269 |
| *iso* C16:0 | 0.210 | 0.197$^{a}$ | 0.198$^{a}$ | 0.210$^{ab}$ | 0.222$^{b}$ | 0.223$^{b}$ | 0.013 | * | 0.210 | * | 0.202 | 0.017 | † | 0.184 | * |
| C17:0 | 0.589 | 0.527$^{a}$ | 0.546$^{ab}$ | 0.566$^{b}$ | 0.616$^{c}$ | 0.689$^{d}$ | 0.016 | *** | 0.563 | * | 0.753 | 0.031 | ** | 0.557 | * |
| C17:1 *cis*-9 | 0.192 | 0.180$^{a}$ | 0.187$^{a}$ | 0.187$^{a}$ | 0.187$^{a}$ | 0.220$^{b}$ | 0.010 | *** | 0.842 | * | 0.232 | 0.017 | 0.414 | 0.309 | † |
| C17:0 + C17:1 *cis*-9 | 0.781 | 0.707$^{a}$ | 0.733$^{ab}$ | 0.753$^{b}$ | 0.802$^{c}$ | 0.908$^{d}$ | 0.023 | *** | 0.759 | * | 0.985 | 0.047 | * | 0.452 | † |
| C17:1 *cis*-9/C17:0 | 0.328 | 0.341$^{b}$ | 0.343$^{b}$ | 0.331$^{ab}$ | 0.305$^{a}$ | 0.317$^{ab}$ | 0.015 | * | 0.611 | * | 0.303 | 0.013 | 0.379 | 0.220 | 0.33 |
| Day 7 | | | | | | | | | | | 0.031 | | | | |
| *iso* C13:0 | 0.030[3] | 0.026$^{a}$ | 0.028$^{a}$ | 0.031$^{b}$ | 0.032$^{b}$ | 0.032$^{b}$ | 0.001 | *** | † | | 0.129 | 0.002 | 0.752 | 0.274 | 0.198 |
| *anteiso* C13:0 | 0.128[3] | 0.107$^{a}$ | 0.114$^{ab}$ | 0.121$^{b}$ | 0.144$^{c}$ | 0.154$^{d}$ | 0.007 | *** | 0.895 | * | 0.168[3] | 0.008 | † | 0.964 | 0.123 |
| *iso* C14:0 | 0.078 | 0.077$^{ab}$ | 0.077$^{ab}$ | 0.083$^{b}$ | 0.081$^{b}$ | 0.073$^{a}$ | 0.005 | * | 0.669 | * | 0.070 | 0.006 | 0.501 | 0.590 | * |
| C15:0 | 1.19 | 1.04$^{a}$ | 1.06$^{a}$ | 1.08$^{a}$ | 1.30$^{b}$ | 1.45$^{b}$ | 0.074 | *** | 0.660 | † | 1.52 | 0.136 | 0.541 | 0.575 | † |
| *iso* C15:0 | 0.222[3] | 0.217$^{a}$ | 0.218$^{a}$ | 0.228$^{bc}$ | 0.229$^{c}$ | 0.219$^{ab}$ | 0.007 | * | 0.425 | * | 0.229[3] | 0.009 | 0.284 | 0.419 | 0.159 |
| *anteiso* C15:0 | 0.453[3] | 0.472$^{b}$ | 0.448$^{a}$ | 0.449$^{a}$ | 0.455$^{ab}$ | 0.439$^{a}$ | 0.012 | * | 0.704 | * | 0.482[3] | 0.015 | * | 0.125 | 0.451 |
| *iso* C16:0 | 0.194[3] | 0.179$^{a}$ | 0.177$^{a}$ | 0.217$^{c}$ | 0.204$^{bc}$ | 0.192$^{ab}$ | 0.013 | ** | 0.211 | * | 0.184[3] | 0.017 | 0.387 | 0.237 | * |
| C17:0 | 0.439[3] | 0.398$^{a}$ | 0.408$^{a}$ | 0.426$^{ab}$ | 0.463$^{b}$ | 0.503$^{c}$ | 0.019 | *** | 0.898 | † | 0.553 | 0.038[3] | † | 0.715 | † |
| C17:1 *cis*-9 | 0.260[3] | 0.229$^{a}$ | 0.238$^{a}$ | 0.237$^{a}$ | 0.285$^{b}$ | 0.310$^{b}$ | 0.016 | *** | 0.673 | * | | 0.028 | 0.135 | 0.459 | * |
| C17:0 + C17:1 *cis*-9 | 0.699[3] | 0.627$^{a}$ | 0.647$^{a}$ | 0.663$^{a}$ | 0.748$^{b}$ | 0.813$^{c}$ | 0.032 | *** | 0.757 | † | 0.889 | 0.064 | † | 0.591 | * |
| C17:1 *cis*-9/C17:0 | 0.592[3] | 0.577$^{ab}$ | 0.585$^{ab}$ | 0.559$^{a}$ | 0.620$^{b}$ | 0.619$^{b}$ | 0.031 | † | 0.637 | * | 0.606[3] | 0.027 | 0.699 | 0.255 | 0.424 |

$^{a–c}$Means within a row in induction wk 1 to 5 with different superscripts differ ($P < 0.05$).

[1]*P*-values according to the linear mixed model for wk 1 to 5 and for wk 5 to 6. Analysis performed with SPSS 15.0.0 (SPSS Inc., Chicago, IL). $Y_{ijk} = \mu + A_i + B_j + C_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, and $\varepsilon_{ijk}$ = residual error term.

[2]Trt = treatment.

[3]Significant difference between d 2 and 7 either over induction wk 1 to 5 (mean) or in wk 6 ($P < 0.05$) according to the linear mixed models (SPSS 15.0.0). Induction wk 1 to 5: $Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + \varepsilon_{ijkl}$, where $Y_{ijkl}$ = dependent variable, $\mu$ = mean, $A_i$ = fixed effect of induction week, $B_j$ = fixed effect of treatment group, $C_k$ = random animal effect, $D_l$ = fixed effect of day, and $\varepsilon_{ijkl}$ = residual error term. Week 6: $Y_{ijk} = \mu + B_i + C_j + D_k + \varepsilon_{ijk}$, where $Y_{ijk}$ = dependent variable, $\mu$ = mean, $B_i$ = fixed effect of treatment group, $C_j$ = random animal effect, $D_k$ = fixed effect of day, and $\varepsilon_{ijk}$ = residual error term.

*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$; †$0.05 < P < 0.10$.

was reported by Vlaeminck et al. (2006a). This was based on observations after at least 2 wk of adaptation. Overall, PCA confirmed formerly reported correlations between rumen VFA proportions and milk OBCFA. However, sudden changes in the rumen microbial environment show no correlation between VFA proportions and milk OBCFA (e.g., in the current protocol an increase in propionate proportions was observed on d 2 of the second week, without a concomitant increase in milk C15:0 and C17:0). This suggests that secretion of these FA is not precursor-driven, contrary to Enjalbert et al. (2008), who linked increases in linear odd-chained fatty acids to increases in their precursor (propionate). Our results seem more in line with French and Armentano (2009) and Vlaeminck et al. (2006a), suggesting that changes in the rumen of OBCFA are determined by changes of the microbial community.

### *Changes in Milk OBCFA and Rumen Parameters During Switch to Control Diet in Wk 6*

After returning to a control diet with less concentrate in wk 6, pH parameters returned to their original values, which confirms observations in the experiment of Enjalbert et al. (2008). However, VFA proportions and milk OBCFA largely follow the same trend as during the induction protocol. Apparently, these parameters require a longer time to return to the original values.

### *Markers of SARA: PCA*

A PCA was performed to visualize the variability in the data, the correlations among different parameters, and the cow-related variation compared with the variation induced by the induction protocol. The FA that

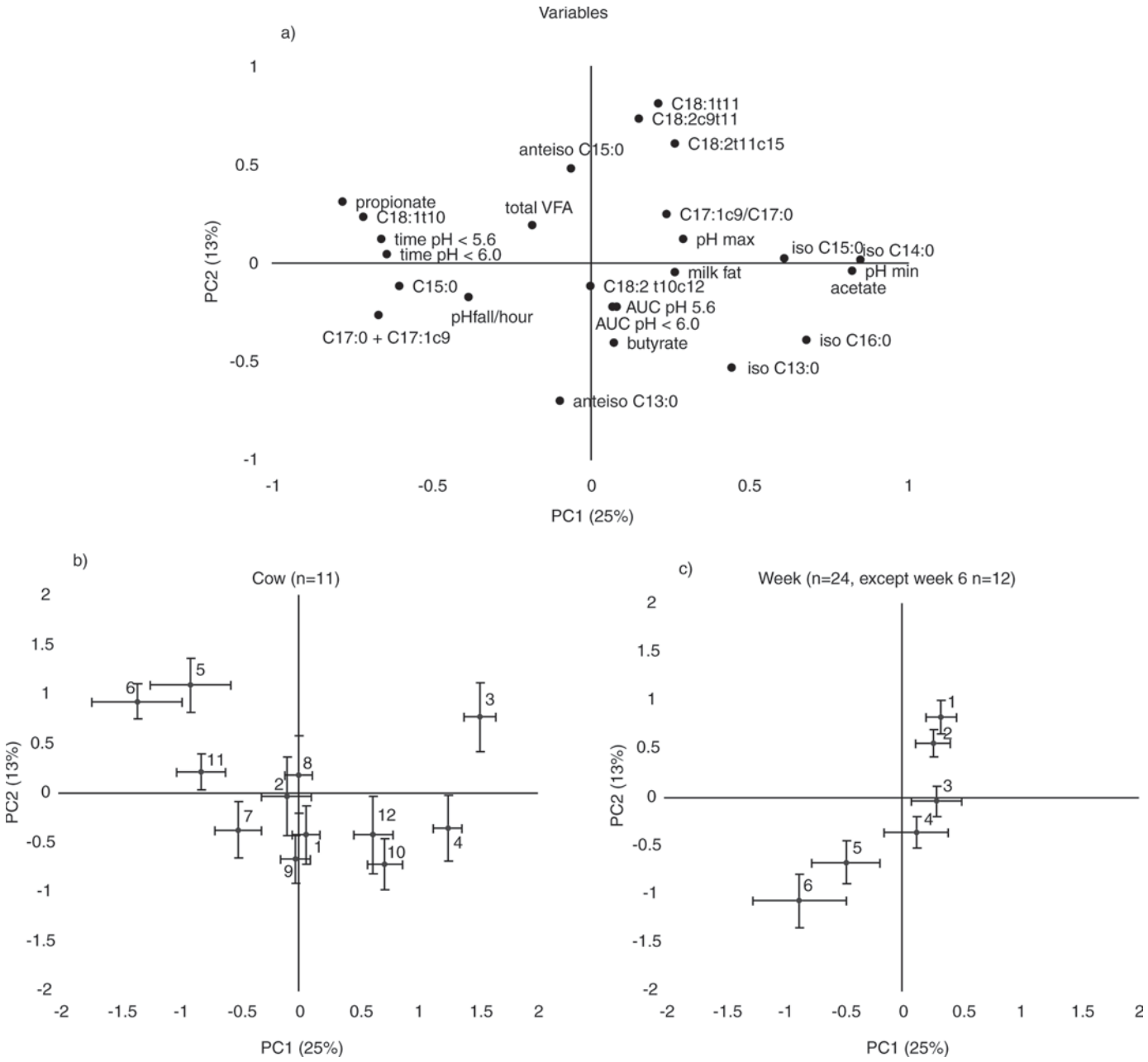


**Figure 3.** Results of a principal component analysis based on rumen parameters [pH minimum (pH min); pH maximum (pH max); pH decrease/hour (pHfall/hour); area under curve (AUC) pH <5.6 or <6.0; time pH <5.6 or <6.0; acetate, butyrate, and propionate proportions; total VFA], fatty acid proportions in milk fat (wt %; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *iso* C16:0, C17:0 + C17:1 *cis*-9, C17:1 *cis*-9/C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *trans*-11,*cis*-15), and milk fat percentage (milk fat) presented as (a) a loading plot of the different variables, (b) a score plot of 12 cows (11 observations/cow), and (c) a score plot of 6 wk, with increasing proportions of wheat-based concentrate in wk 1 to 5 and increased concentrate amounts in wk 6 (24 observations/wk, except for wk 6, which includes 12 observations). In the score plots, means and standard errors of principal components (PC) 1 and 2 are presented.

had the highest but opposite loadings on PC1 were C18:1 *trans*-10, C15:0, C17:0 + C17:1 *cis*-9, and *iso* C14:0 (Figure 3). Strikingly, cow differences rather than induction protocol were mainly associated with PC1. This suggests that rumen acidosis parameters such as time pH <5.6 and minimum pH were determined more by cow than by induction protocol, as also described in a review by Beauchemin and Penner (2009). Similarly, cows differed largely in milk fat *iso* C14:0 and C18:1 *trans*-10 concentrations.

Scores of the first 4 wk were spread along PC2. It is not surprising that averages of wk 1 to 4 did not vary

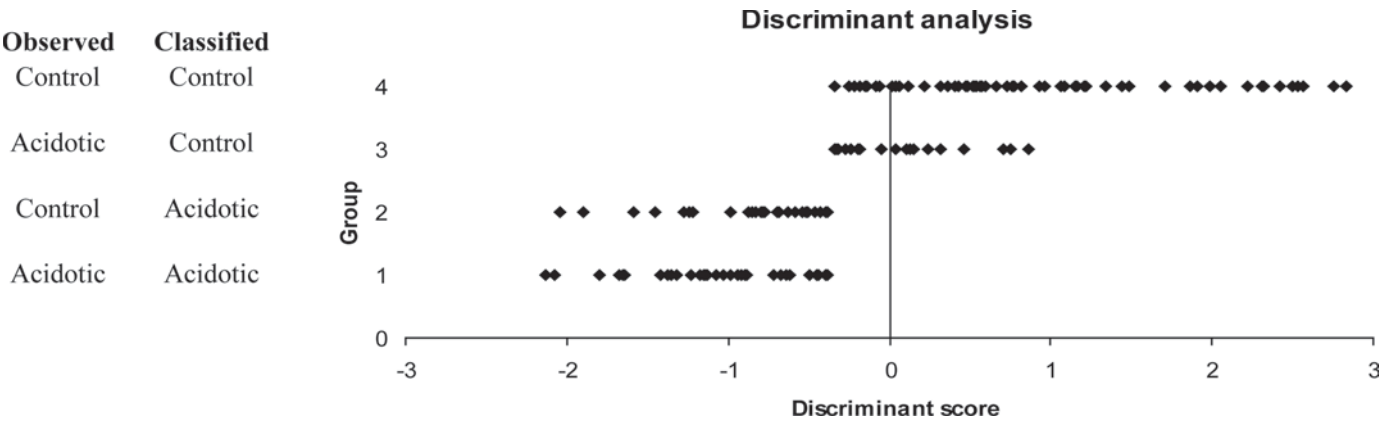


**Figure 4.** Score plot of a stepwise discriminant analysis, including fatty acid proportions in milk fat (wt %; *anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *iso* C16:0, C17:0 + C17:1 *cis*-9, C17:1 *cis*-9/C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, and C18:2 *trans*-11, *cis*-15), milk fat percentage, and proportional (ratio) or differential changes (diff) compared with wk 1 (ratio C18 *trans*-10, diff C18 *trans*-10, ratio *iso* C14, and diff *iso* C14).

along PC1 because no major induction of SARA (except for a temporary pH decrease on d 2 of the second week) occurred during the first 4 wk. Evolution toward acidosis in wk 5 and 6 was associated with a deviation from PC2 to the negative PC1 axis. Other milk parameters, reported in literature to be indicative of rumen acidosis, are reduction in milk yield and fat content and increase in protein concentration (e.g., Plaizier et al., 2008). Although this was confirmed in our induction experiment, PCA revealed that the link between milk fat was less correlated than specific milk FA to rumen acidosis parameters.

### *Markers of SARA: DA*

Discriminant analysis was performed in a first attempt to distinguish acidotic from nonacidotic cows based on the milk FA pattern and milk fat content. After cross-validation, only 65.2% of the cases were correctly classified, and classification was based mainly on C18:2 *cis*-9,*trans*-11, *iso* C16:0, and *iso* C13:0. This result confirmed the deviation from PC2 to PC1 because those FA are related to PC1 as well as PC2. These FA originate from the de novo synthesis by the microbial population or from the ruminal biohydrogenation to C18:1 *trans*-11, which then partially is desaturated to *cis*-9,*trans*-11 conjugated linoleic acid in the mammary gland (Shingfield et al., 2007). As already confirmed in the literature, this means that subacute acidosis affects the ruminal environment, resulting in changes of both the nature of the ruminal microbial population and the extent of biohydrogenation, or its pattern, or both. Strikingly, biohydrogenation intermediates that have been suggested before to be associated with rumen acidosis (e.g., C18:1 *trans*-10 and C18:2 *trans*-10,*cis*-12) were not selected as the most discriminating milk FA in this experiment. Also, milk fat reduction or content, which have been used in some experiments to indicate lack of dietary fiber (structure) and acidosis risk (De Brabander et al., 1999), have not come forward in this experiment as a predominant discriminator.

Because most incorrectly classified cases (i.e., false control and false acidotic) are located between both correctly classified groups, these cases are probably on the border of suffering from acidosis or not being acidotic. Hence, classification in continuous probability classes indicating the risk of acidosis development will be considered in future work. For this purpose, other classification algorithms from the field of machine learning will be applied.

## CONCLUSIONS

As expected, traditional markers of SARA (rumen pH, VFA, milk fat, DMI) responded broadly to an increase in fermentable carbohydrate. Most of the milk

**Table 9.** Classification results of a stepwise discriminant analysis with cross-validation including milk fatty acids[1,2]

| | | Predicted membership[3] | | |
|---|---|---|---|---|
| Item | Acidosis | 0 | 1 | Total |
| Count | 0 | 59 | 27 | 86 |
| | 1 | 21 | 31 | 52 |
| Percentage | 0 | 68.6 | 31.4 | 100 |
| | 1 | 40.4 | 59.6 | 100 |

[1]*Anteiso* C13:0, *iso* C13:0, *iso* C14:0, C15:0, *iso* C15:0, *iso* C16:0, C17:0 + C17:1 *cis*-9, C17:1 *cis*-9/C17:0, C18:1 *trans*-10, C18:1 *trans*-11, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, C18:2 *trans*-11,*cis*-15, milk fat percentage, ratio C18 *trans*-10, difference C18 *trans*-10, ratio *iso* C14, and difference *iso* C14.

[2]Only 65.2% of grouped cases were correctly classified.

[3]Where 0 = nonacidotic, 1 = acidotic.

FA, including OBCFA, showed a response that was expected based on the rumen fermentation pattern during the induction experiment. Milk fat, a regularly used parameter indicative of SARA, showed a weaker link with rumen acidosis parameters than specific milk FA. The most effective predictors in milk fat of low rumen pH were C18:2 *cis*-9,*trans*-11, *iso* C16:0, and *iso* C13:0. This shows that specific milk FA have potential value in identifying cows at risk of acidosis.

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## REFERENCES

AlZahal, O., E. Kebreab, J. France, and B. W. McBride. 2007. A mathematical approach to predicting biological values from ruminal pH measurements. J. Dairy Sci. 90:3777–3785.

AOCS. 2004. Am 5–04: Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. Amercian Oil Chemists' Society, Urbana, IL.

Beauchemin, K., and G. Penner. 2009. New developments in understanding ruminal acidosis in dairy cows. Pages 6–17 in Proc. 18th Annual Tri-State Dairy Nutrition Conference. Ohio State University Dept. of Animal Science, Columbus.

Boeckaert, C., B. Vlaeminck, J. Dijkstra, A. Issa-Zacharia, T. Van Nespen, W. Van Straalen, and V. Fievez. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition. J. Dairy Sci. 91:4714–4727.

Cabrita, A. R. J., R. J. B. Bessa, S. P. Alves, R. J. Dewhurst, and A. J. M. Fonseca. 2007. Effects of dietary protein and starch on intake, milk production, and milk fatty acid profiles of dairy cows fed corn silage-based diets. J. Dairy Sci. 90:1429–1439.

Conway, E. J. 1962. Microdiffusion analysis and volumetric error. 5th ed. Crosby Lockwood, London, UK.

Craninx, M., V. Fievez, B. Vlaeminck, and B. De Baets. 2008. Artificial neural network models of the rumen fermentation pattern in dairy cattle. Comput. Electron. Agric. 60:226–238.

CVB. 2007. Veevoedertabel (Table of Nutritional Value of Feed Ingredients). Centraal Veevoederbureau, Lelystad, the Netherlands.

De Brabander, D. L., J. L. DeBoever, J. M. Vanacker, C. V. Boucque, and S. M. Botterman. 1999. Evaluation of physical structure in dairy cattle nutrition. Pages 111–145 in Proc. Recent Advances in Animal Nutrition. P. C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.

Dragomir, C., D. Sauvant, J.-L. Peyraud, S. Giger-Reverdin, and B. Michalet-Doreau. 2008. Meta-analysis of 0 to 8h post-prandial evolution of ruminal pH. Animal 10:1437–1448.

Duffield, T., J. C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59–66.

Enemark, J. M. D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176:32–43.

Enjalbert, F., Y. Videau, M. C. Nicot, and A. Troegeler-Meynadier. 2008. Effects of induced subacute ruminal acidosis on milk fat content and milk fatty acid profile. J. Anim. Physiol. Anim. Nutr. (Berl.) 92:284–291.

French, E. A., and L. E. Armentano. 2009. Effects of infusing volatile fatty acids intraruminally on rumen and milk odd and branched chain fatty acids. J. Dairy Sci. 92(E-Suppl. 1):458–459.

Hindle, V. A., A. M. van Vuuren, A. Klop, A. A. Mathijssen-Kamman, A. H. van Gelder, and J. W. Cone. 2005. Site and extent of starch degradation in the dairy cow—A comparison between in vivo, in situ and in vitro measurements. J. Anim. Physiol. Anim. Nutr. (Berl.) 89:158–165.

ISO. 1999. ISO 6496—Animal feeding stuffs—Determination of moisture and other volatile matter content. International Organisation for Standardisation, Geneva, Switzerland.

ISO. 2002. ISO 5984—Animal feeding stuffs—Determination of crude ash. International Organisation for Standardisation, Geneva, Switzerland.

ISO. 2006. ISO 3889—Milk and milk products—specification of Mojonnier-type fat extraction flasks. International Organisation for Standardisation, Geneva, Switzerland.

ISO. 2008. ISO 16634—Food products—Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content. International Organisation for Standardisation, Geneva, Switzerland.

Keunen, J. E., J. C. Plaizier, S. L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J. Dairy Sci. 85:3304–3313.

Kramer, J. K. G., M. Hernandez, C. Cruz-Hernandez, J. Kraft, and M. E. R. Dugan. 2008. Combining results of two GC separations partly achieves determination of all *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-Ion SPE fractionation. Lipids 43:259–273.

Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2009. Subacute ruminal acidosis in dairy cows: the physiological causes, incidences and consequences. Vet. J. 176:21–31.

Raes, K., S. De Smet, and D. Demeyer. 2001. Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Anim. Sci. 73:253–260.

Shingfield, K. J., S. Ahvenjarvi, V. Toivonen, A. Vanhatalo, and P. Huhtanen. 2007. Transfer of absorbed *cis*-9, *trans*-11 conjugated linoleic acid into milk is biologically more efficient than endogenous synthesis from absorbed vaccenic acid in lactating cows. J. Nutr. 137:1154–1160.

Sun, Y. Z., S. Y. Mao, and W. Y. Zhu. 2010. Rumen chemical and bacterial changes during stepwise adaptation to a high-concentrate diet in goats. Animal 4:210–217.

Uden, P., P. H. Robinson, and J. Wiseman. 2005. Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. Anim. Feed Sci. Technol. 118:181–186.

Van Ranst, G., V. Fievez, M. Vandewalle, C. Van Waes, J. De Riek, and E. Van Bockstaele. 2010. Influence of damaging and wilting red clover on lipid metabolism during ensiling and in vitro rumen incubation. Animal doi:10.1017/S1751731110000625

Vlaeminck, B., C. Dufour, A. M. Van Vuuren, A. M. R. Cabrita, R. J. Dewhurst, D. Demeyer, and V. Fievez. 2005. Potential of odd and branched chain fatty acids as microbial markers: Evaluation in rumen contents and milk. J. Dairy Sci. 88:1031–1042.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006a. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389–417.

Vlaeminck, B., V. Fievez, S. Tamminga, R. J. Dewhurst, A. Van Vuuren, D. De Brabander, and D. Demeyer. 2006b. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. J. Dairy Sci. 89:3954–3964.

Zaaijer, D, W. D. J. Kremer, and J. P. T. M. Noordhuizen. 2001. Dairy cow monitoring in relation to fertility performance. Scoring cards. Pharmacia Animal Health, UK